Full Title: The impact of grazing on seedling patterns in degraded sparse-elm grassland

Short Title: Seedling patterns affected by grazing

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#### ABSTRACT

Over-grazing by livestock in semi-arid ecosystems is one of the main causes of desertification. Although over-grazing presents a global environmental challenge, only a few studies have investigated grazing impacts on the composition of species and functional groups of seedling bank. In this study, we determined whether the composition of seedling species and functional groups, as well as the correlations between the seedlings of sparse-elm (*Ulmus pumila* var. *sabulosa* or *U. pumila*) and other species changed under three grazing intensities in the degraded sparse-elm grassland in the Horqin Sandy Land, China. Species composition and abundance of established seedlings were surveyed and the relationships between seedlings of *U. pumila* and other species were analyzed. The results showed that plant communities under moderate grazing were more stable than the other two grazing intensities due to higher seedling density, higher species richness and higher number of perennial herbs. Seedlings of *U. pumila* could even prevent noxious seedlings growth under moderate grazing. We concluded that moderate grazing could benefit the recovery of this sparse-elm grassland in the Horqin Sandy Land.

**KEYWORDS:** Horqin Sandy Land, semi-arid ecosystem; seedling bank; grazing intensity; species composition

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#### **INTRODUCTION**

Sparse forest steppe is a community type composed of sparse trees, shrubs and herbaceous species, mainly distributed in tropics, subtropics and temperate regions. It is important to maintain global water-thermal balance (Dulamsuren *et al.*, 2009). In China, the temperate sparse forest steppe is mainly distributed in the Horqin Sandy Land, with sparse-elm grassland as the climax community. Vegetation in sparse-elm grassland is dominated by elm (*Ulmus pumila* var. *sabulosa* or *U. pumila*), the only tree species in this system. As the dominant species in sparse-elm grassland, elm trees play an irreplaceable role in enhancing biodiversity and ecosystem stability (Yu and Chen, 2007; Li *et al.*, 2011), reducing soil erosion, and stabilizing sand dunes (Liu *et al.*, 2013).

In the Horqin Sandy Land, low rate of seed germination and seedling survival often inhibit plant natural regeneration (Zuo *et al.*, 2005; Liu *et al.*, 2012). Previous studies indicated that seedling bank is critical in shaping plant populations and community formation (Hara, 1987; Chen *et al.*, 2014). To understand the demographic processes and growth of seedling banks, it is important to recognize the structure of seedling bank and the interactions among seedlings (Li *et al.*, 2010a). Seedling stage represents a demographic bottleneck for most populations, particularly for species that do not have clonal ability (Messaoud and Houle, 2006; Li *et al.*, 2010a). High rate of seedling mortality, mainly caused by competition, is one factor that affects the selection pressures during seedling stage in this grassland community (Ooi et al., 2014), which in part, contributes to the low densities of most species (Cruz-Rodriguez and Lopez-Mata, 2004). During target plant growing period, competition for resources does not occur between all plants in the population, but rather between plants immediately surround the individual target plant (Florentine and Fox, 2003; Smith and Hunsley, 2006). In semi-arid ecosystems, the intensity of resource competition among tree, shrub and grass seedlings (e.g., competition between *U. pumila* and grass seedlings) is dependent on the number of neighbors

(Florentine and Fox, 2003). Consequently, interaction between species plays a significant role in nature regeneration and species coexistence in communities of degraded sparse-elm grassland. While both interspecific and intraspecific competition may be detrimental for seed germination and seedling survival (Florentine and Fox, 2003; Smith and Hunsley, 2006), studies also showed that seedling interactions can be mutually beneficial and may accelerate the recovery of sparse-elm in Sandy Land (Harris *et al.*, 2006; Nichols *et al.*, 2010). Therefore, how seedling interactions influence community composition in this ecosystem, either by facilitation or competition, are worth investigating (Nichols *et al.*, 2010).

Apart from competition, livestock grazing also affects the process of species regeneration due to its immediate impacts (e.g., gnawing and trampling) on seed germination and seedling survival (Dulamsuren et al., 2009; Tsegaye et al., 2009), as well as its indirect influences on soil texture, soil seed bank and vegetation coverage (Wang et al., 2009; Li et al., 2010b; Daryanto et al., 2013). Since livestock grazing is the most important grass removal agent in the Horqin Sandy Land (Xu and Ning, 2010), its impacts on the natural regeneration of vegetation community in this degraded sparse-elm grassland are essential to better understand the vegetation dynamics in this region. Interactions between seedling competition and grazing, both positive and negative, are important determinants of community renewal in degraded sparse-elm grassland (Nichols et al., 2010; Wang et al., 2016). A better understanding of the role of interactions will benefit restoration in this area because it will help explain and predict the adult plant populations (Nathan et al., 2000). So far, studies that focus on the interactions among herb-shrub-tree have been mostly in the savannas (Riginos and Young, 2007; Scheiter and Higgins, 2007; Wang et al., 2010), rather than in temperate grasslands. To address such knowledge gaps, we addressed three sets of questions in this study: (1) Does the seedling composition change along grazing intensity gradient? (2) Does the functional group of seedling bank change along grazing intensity gradient? (3) How does

the relationship between elm (*U. pumila*) seedlings and other seedlings change along grazing intensity gradient?

# MATERIAL AND METHODS

# Study area

The study was conducted at the Wulanaodu region  $(42^{\circ}29'-43^{\circ}06'\text{N}, 119^{\circ}39'-120^{\circ}02'\text{E},$ altitude approx. 480 m) in the south-western Horqin Sandy Land, Inner Mongolia, China. The study area is classified as semiarid climate. The average annual temperature is 6.3°C and the frost-free period extends about 130 days. The coldest and hottest months are January and July, respectively. The annual average precipitation is 340.5 mm, 70% of which falls between June and September. Average annual wind velocity varies between 3.2 and 4.5 m s<sup>-1</sup>. The dominant wind direction is from the north-west in March-May and the south-west in June-September. Sparse-elm grassland is the climax successional community in the Horqin Sandy Land, China. It is characterized by single tree species (*U. pumila*) and well-developed grass-shrub vegetation, being similar to subtropical sparse woodland savanna.

### Experimental design and data collection

To understand the correlations between *U. pumila* seedlings and other seedlings along a grazing gradient, ten  $1 \text{ m} \times 1$  m quadrats were randomly set up in each sparse-elm grassland, which have experienced different grazing intensities during the last 20 years: light grazing sparse-elm grassland (LG); moderate grazing (MG) sparse-elm grassland; and heavy grazing sparse-elm grassland (HG) (Table 1). These grazing intensities (sheep units ha<sup>-1</sup>) were represented based on the stocking rates (= livestock (sheep) numbers/pasture area) according to the method of Wu et al. (2015) because the three grazing-level plots were set up on different sheep ranches. They were all situated in freely grazed meadows and owned by different families. Mean stocking rates and vegetation cover in each meadow are given in

Table 1. Species composition and abundance of seedlings bank were recorded in each quadrat in the early June 2015. We classified all plant species to three plant functional groups as edible grass species group, inedible grass species group and noxious grass species group. *U. pumila* is not noxious and can be consumed by the livestock and therefore it is not a problem species (Table 2).

# Statistical analyses

Differences in the functional groups of seedling bank along a different grazing intensity were considered as significant at a level of P < 0.05 with the LSD test (Wu *et al.*, 2009; Wang *et al.*, 2015). All statistical analyses were performed with the SPSS 16.0. software. Detrended correspondence analysis (DCA) (Plassmann *et al.*, 2009; Yassir *et al.*, 2010) was used to examine species composition under three different grazing intensities. Principal Component Analysis (PCA) was used to examine the relationship between seedlings of *U. pumila* and other species under three different grazing intensities. DCA and PCA were performed with CANOCO 4.5 software, using the default settings in CANOCO in all analysis.

# RESULTS

#### Species composition of seedling bank

There were 23 species recorded in the seedling bank (Table 2). Total species richness decreased from LG to HG, with 17, 13 and 8 species being recorded under LG, MG and HG, respectively (Fig. 1A). There was a higher proportion of annual herb species in the seedling bank of LG and HG, while a higher proportion of perennial herb species was found in the seedling bank of MG (Fig. 1A). The average seedling density under MG was higher than those under other two grazing intensities (P < 0.05), with more perennial herb seedlings under LG and MG, but more annual herb seedlings under HG (Fig. 1B).

#### Functional groups of seedling bank

The average total seedling density of *U. pumila* increased along the grazing gradient from LG to HG (Table 3). The percentage of *U. pumila* seedlings under HG was significantly higher than those under other two grazing intensities (P < 0.05) (Table 3). The highest percentage of noxious species (*Diarthron linifolium*) occurred under HG, while the lowest percentage was found under MG. The percentage of inedible grass species group, however, was similar between different grazing intensities (Table 3).

#### Correlation between seedlings of U. pumila and other species

The seedling bank showed a significant clustering along the first DCA axis, which was significantly correlated with grazing intensities. The highest dissimilarity was found between MG and HG (Fig. 2). There was a considerable overlap in community composition between LG and MG, compared to HG. Under each grazing level, *U. pumila* seedlings were correlated differently with different species. Under LG, there were positive correlations between *U. pumila* seedlings and *C. glaucum, C. acuminatum, T. terrestris, L. echinata, O. racemosa, C. candelabrum, B. dasyphylla, L. bicolor, C. duriuscula, D. linifolium, T. berteronianus, S. viridis, E. brachystachyus, A. odorum, but negative correlations between <i>U. pumila* seedlings were positively correlated with *S. tabernaemontani, C. duriuscula, I. lacteal, and P. centrasiaticum, negatively* correlated with *G. sibiricum, C. squarrosa, D. linifolium, S. ruthenica, L. bicolor, C. annannii, A. scoparia,* but had no significant correlation *U. pumila* with *A. odorum* (Fig. 4). Under HG, the seedlings of *U. pumila* were positively correlated with *D. linifolium, T. terrestris, C. duriuscula,* and *S. viridis.* There was, however, negative correlations between *U. pumila* seedlings between *U. pumila* seedlings of *U. pumila* were positively correlated with *D. linifolium, T. terrestris, C. duriuscula,* and *S. viridis.* There was, however, negative correlations between *U. pumila* seedlings between *U. pumila* seedlings and *C. candelabrum, L. echinata,* and *E. brachystachyus* (Fig. 5).

#### DISCUSSION

### Species composition response to grazing

Our data suggested that grazing disturbance affected the average seedling density, and there was a shift in total species richness (abundance) as a response to grazing gradient in the studied degraded sparse-elm grassland. These data supported findings from other grasslands where grazing is a common practice (Zhu et al., 2012; Alvarez-Martinez et al., 2016; Wu et al., 2016). In a warm humid summer climate (i.e., Cfa according to Köppen classification system) where many integrated crop-livestock systems are found, grazing intensity is directly linked to vegetation community structure (Schuster et al., 2016). Accordingly, grazing intensity greatly influences plant community characteristics (Wu et al., 2016; Waters et al., 2017). The average seedling density under HG was low. Different from MG, which was dominated by perennial herbs, HG had annual herbs as the dominant life form (Fig. 1). Under HG, vegetation over-consumption and long seed production cycle of perennial herbs reduced their abundance. In contrast, annual herb species usually have small seeds (Moles et al., 2004), which can easily enter into the soil to form soil seed bank. Annual plants can also produce more seeds per unit ground area than large-seeded species, giving them an advantage over perennial plants during seed production (Moles et al., 2004). Our study thus showed that smaller-seeded species was benefited over the larger-seeded species with increasing levels of grazing, similar to the study by Wu et al. (2015) in the alpine meadow of China. These results indicated that plant communities under HG might be less stable due to low average seedling density, as well as low species and low number of perennial herb.

# Functional groups response to grazing

Grazing significantly affects community functional trait assembly due to its selective effects on the species individual performance (Wu *et al.*, 2014). Our study has shown that the highest percentage of noxious grass species group was found under HG, but it was lowest under MG. Strong competition from perennial herb species that was found under MG could contribute to lower percentage of noxious weeds which was dominated by annual herb species in our study site. Other study showed that increasing grazing pressure changes chemical and physical properties of topsoil, enhancing soil degradation processes, and eventually affecting the structure of vegetation (Kölbl *et al.*, 2011). Grazing can stimulate growth of some grazing-tolerant plant species and contribute to changes in species diversity, and productivity (Augustine and McNaughton 1998; Frank *et al.*, 2014). This result supported the concept that grazing had a significant effect on the contribution of some functional groups shaping the structure of vegetation (Bermejo *et al.*, 2012).

Our result also indicated that the percentage and the density of *U. pumila* seedling were highest under HG. A higher percentage of *U. pumila* seedlings under HG, however, was due to the reduction of the total seedling pool number, particularly that of perennial grasses, rather than a higher number of *U. pumila* seedlings itself.

#### Correlation responses to grazing

Our results suggested that seedling bank species composition was significantly correlated with grazing intensities, as shown along the first DCA axis, which explained 71.8% of variation in species composition (Fig. 2). Since species composition was relatively clustered under MG than under LG, our result indicated that plant communities under MG were more stable than LG and HG. This statement was also supported by higher seedling density under MG than either LG or HG, and MG domination by perennial herbs (Fig. 1). Indeed, the dominance of perennial tussock grasses has been recorded to stabilize the ecosystem functions under moderate grazing in abandoned grasslands with a homogeneous vegetation structure (Ockinger *et al.*, 2006; Moinardeau *et al.*, 2016).

We also highlighted the importance of defining grazing intensity when studying the impacts of grazing. For example, a study by Qu *et al.* (2016) showed that light grazing,

instead of moderate grazing markedly increase the Shannon-Wiener index and Pielou evenness index in Jilin province, China under semiarid climate. In fact, the different conclusions are related to the different definitions in grazing intensity. In our study, we defined the grazing intensity as 0.76 sheep units ha<sup>-1</sup> for LG, 1.88 sheep units ha<sup>-1</sup> for MG, and 2.68 sheep units ha<sup>-1</sup> for HG while in Qu et al. (2016), they defined the grazing intensity as 0 sheep units ha<sup>-1</sup> for control, 4 sheep units ha<sup>-1</sup> for LG, 6 sheep units ha<sup>-1</sup> for HG.

Overall, the seedlings of *U. pumila* and other species under different grazing intensities showed different correlations. There was a positive correlation between the seedlings of *U. pumila* and the vast majority species under LG. We postulated that due to low seedling density and light grazing disturbance, there was less interspecific competition. On the other hand, there was negative correlation between the seedlings of *U. pumila* and the vast majority species under MG. As total seedling density increased from LG to MG (Fig. 1), there could be more interspecific competition between the seedlings of *U. pumila* and the other species under MG. Due to the same line of reasoning, our result suggested that *U. pumila* seedlings could prevent noxious seedlings growth under MG. Our data also showed both positive correlations and negative correlations between the seedling density and led to less interspecific competition, it also deteriorated the soil chemical and physical properties, which in turn increased species competition. Therefore, it is unsurprising if we found a mix result under HG in our study area.

# CONCLUSIONS

The results of our study showed that plant communities under MG were more stable due to higher average seedling density, higher species richness and higher number of perennial herbs. Since *U. pumila* seedlings could also prevent noxious seedlings growth under MG, we suggested that MG (1.88 sheep units ha<sup>-1</sup>) could be an effective grazing density to help the recovery of degraded sparse-elm grassland in the Horqin Sandy Land. This study provides valuable data towards a better understanding of vegetation dynamics in areas affected by different grazing intensity.

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Table 1. Description of the vegetation and basic study site properties of the investigated three

Grazing intensities	Latitude and	Altitude	Mean stocking rates	Vegetation cover	
Grazing intensities	longitude	(m)	(sheep units ha <sup>-1</sup> )	(%)	
Light grazing	$N43.08272^\circ$ ,	578m	0.76	40	
(LG)	E119.60977°	576111	0.70	40	
Moderate grazing	$N43.08526^\circ$ ,	501m	1.88	20	
(MG)	E119.61262°	301111	1.00	20	
Heavy grazing	$N43.08591^\circ$ ,	516-00	2 69	10	
(HG)	E119.611103°	546m	2.68	10	

degraded sparse-elm grassland with different grazing intensities by sheep.

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Species	Abbreviated code	Edible	Life form	Family
1 Ulmus pumila var. sabul	osa U. pumila	Y	Т	Ulmaceae
2 Carex duriuscula	C. duriuscula	Y	PH	Cyperaceae
3 Lappula echinata	L. echinata	Y	ABH	Boraginaceae
Diarthron linifolium	D. linifolium	N (noxious)	AH	Thymelaeaceae
5 Corispermum candelabr	rum C. candelabrum	Y	AH	Chenopodiaceae
6 Lespedeza bicolor	L. bicolor	Y	S	Leguminosae
7 Bassia dasyphylla	B. dasyphylla	Ν	AH	Chenopodiaceae
8 Oxytropis racemosa Tur	rcz. O. racemosa	Ν	PH	Leguminosae
9 Artemisia scoparia	A. scoparia	Y	ABH	Compositae
10 Chenopodium acuminat	um C. acuminatum	Y	AH	Chenopodiaceae
11 Setaria viridis	S. viridis	Y	AH	Gramineae
12 Tragus berteronianus	T. berteronianus	Y	AH	Gramineae
13 Enneapogon brachystach	nyus E. brachystachyus	Y	AH	Gramineae
14 Tribulus terrestris	T. terrestris	Y	AH	Zygophyllaceae
15 Chenopodium glaucur	n C. glaucum	Y	AH	Chenopodiaceae
16 Convolvulus ammann	ii C. ammannii	Ν	PH	Convolvulaceae
17 Allium odorum	A. odorum	Y	PH	Liliaceae
18 Pennisetum centrasiatic	um P. centrasiaticum	Y	PH	Gramineae
19 Iris lacteal var. chinens	sis I. lacteal	Ν	PH	Iridaceae
20 Scirpus tabernaemonta	ni S. tabernaemontani	Y	PH	Cyperaceae
21 Geranium sibiricum	G. sibiricum	Y	PH	Geraniaceae
22 Cleistogenes squarros	a C. squarrosa	Y	PH	Gramineae
23 Salsola ruthenica	S. ruthenica	Y	AH	Chenopodiaceae

Table 2. Species composition in seedling bank of the sparse-elm grassland. AH = annual herb, ABH = annual-biennial herb, PH = perennial herb, S = shrub, T = tree.

Table 3. Response of plant functional groups in the three degraded sparse-elm grasslands with

Items	Grazing intensities			
nems	LG	MG	HG	
Average total <i>U. pumila</i> seedling density (individual m <sup>-2</sup> )	8.10±1.96a	13.10±4.14a	13.60±6.10a	
The percentage of U. pumila seedling	5.26±1.10a	$4.77 \pm 1.24a$	19.69±3.91b	
The percentage of inedible grass species group	14.83±5.05a	1.94±1.26a	17.57±8.62a	
The percentage of noxious grass species group	10.95±4.58ab	$0.84 \pm 0.84 b$	17.57±8.62a	

different grazing intensities by sheep.

Note: Means with the same letter are not significantly different at p < 0.05. Values are mean  $\pm$  SE.

Accepted

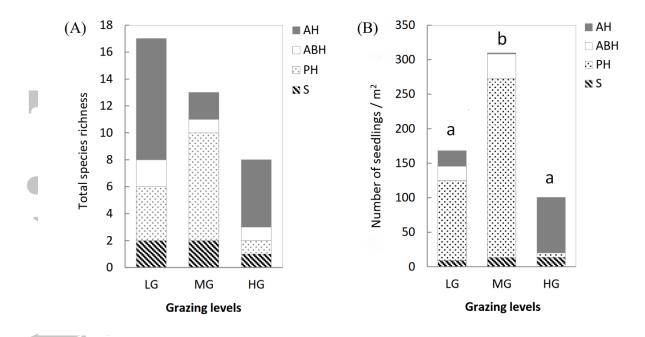


Fig. 1. Total species richness (A) and average seedling density (B) under different grazing intensities. Key to legend codes are: AH = annual herb, ABH = annual-biennial herb, PH = perennial herb, S = shrub.

Accepted

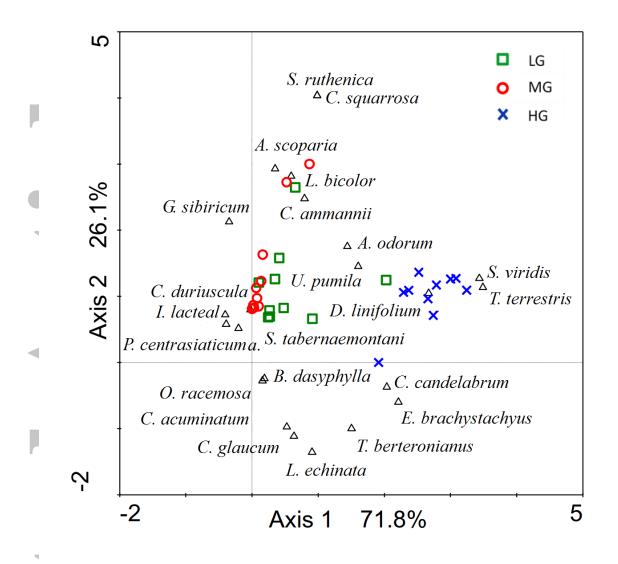


Fig. 2. Detrended Correspondence Analysis (DCA) of species composition under three different grazing intensities. Abbreviations for the variables are given in Table 2.

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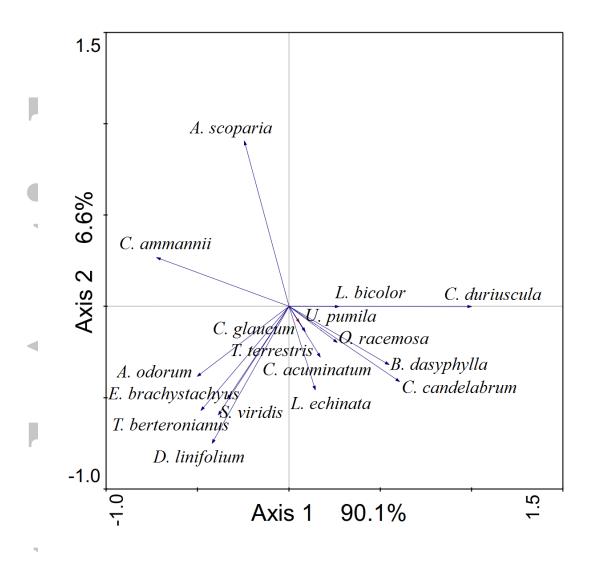


Fig. 3. Principal Component Analysis (PCA) of the relationship between seedlings of *U*. *pumila* and other species under LG. The amount of variability explained by all the canonical axes was 96.7%. Abbreviations for the variables are given in Table 2.

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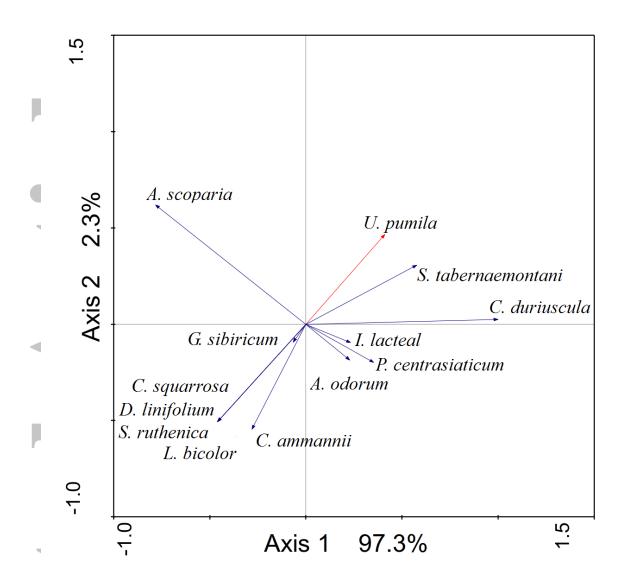


Fig. 4. Principal Component Analysis (PCA) of the relationship between seedlings of *U*. *pumila* and other species under MG. The amount of variability explained by all the canonical axes was 99.6%. Abbreviations for the variables are given in Table 2.

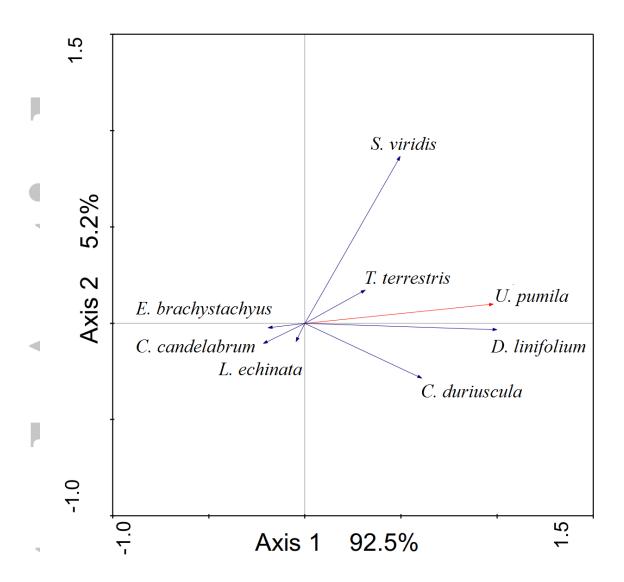


Fig. 5. Principal Component Analysis (PCA) of the relationship between seedlings of *U*. *pumila* and other species under HG. The amount of variability explained by all the canonical axes was 97.7%. Abbreviations for the variables are given in Table 2.